#11 Attachment 10/07/751

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/00, 14/435, 16/18, C12N 1/21, 15/09, 15/12, 15/63, A61K 35/64, 38/16, 39/00, 39/35

A1

(11) International Publication Number: **WO 96/28469**

(43) International Publication Date: 19 September 1996 (19.09.96)

(21) International Application Number: PCT/US96/03133

(22) International Filing Date: 8 March 1996 (08.03.96)

(30) Priority Data:
401,509 9 March 1995 (09.03.95) US

(60) Parent Application or Grant
(63) Related by Continuation
US 401,509 (CIP)
Filed on 9 March 1995 (09.03.95)

(71) Applicant *(for all designated States except US)*: HESKA CORPORATION [US/US]; 1825 Sharp Point Drive, Fort Collins, CO 80525 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: STIEGLER, Gary, L. [US/US]; 2934 Zendt Drive, Fort Collins, CO 80526 (US). RUSHLOW, Keith, E. [US/US]; 1600 Dogwood Court, Fort Collins, CO 80525 (US).

(74) Agents: CONNELL, Gary, J. et al.; Sheridan Ross & McIntosh, Suite 3500, 1700 Lincoln Street, Denver, CO 80203 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: HEMATOPHAGOUS INSECT CALRETICULIN NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF

(57) Abstract

The present invention is directed to novel insect calreticulin nucleic acid molecules and proteins. The present invention includes hematophagous insect nucleic acid molecules that hybridize under stringent hybridization conditions to a flea calreticulin gene as well as hematophagous insect calreticulin proteins encoded by such nucleic acid molecules. The present invention also includes therapeutic compositions capable of reducing insect infestation or allergic dermatitis, as well as methods to treat animals with such therapeutic compositions. Examples of such therapeutic compositions include hematophagous insect calreticulin proteins, nucleic acid molecules encoding such proteins, anti-calreticulin antibodies and calreticulin inhibitors.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

HEMATOPHAGOUS INSECT CALRETICULIN NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to novel hematophagous insect calreticulin nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies to such proteins, inhibitors of such proteins, and uses of therapeutic compositions comprising such nucleic acid molecules, proteins, antibodies and/or inhibitors to protect an animal from insect infestation, both on and in the environment surrounding the animal.

BACKGROUND OF THE INVENTION

Hematophagous (i.e., bloodsucking) insect infestation, and in particular flea infestation, of animals, is a health and economic concern because insects are known to cause and/or transmit a variety of diseases. Insects directly cause a variety of diseases, including allergies, and also carry a variety of infectious agents including, but not limited to, endoparasites (e.g., nematodes, cestodes, trematodes and protozoa), bacteria and viruses. In particular, the bites of insects are a problem for animals maintained as pets because the infestation becomes a source of annoyance not only for the pet but also for the pet owner who may find his or her home generally contaminated with insects. As such, insects are a problem not only when they are on an animal but also when they are in the general environment of the animal.

Bites from fleas are a particular problem because they not only can lead to disease transmission but also can

cause a hypersensitive response in animals which is manifested in a disease called flea allergic (or allergy) dermatitis (FAD). A hypersensitive response in animals typically results in localized tissue inflammation and damage, causing substantial discomfort to the animal.

The medical and veterinary importance of insect infestation has prompted the development of reagents capable of controlling insect infestation. Commonly encountered methods to control insect infestation are generally focussed on use of insecticides in formulations such as sprays, shampoos, dusts, dips, or foams, or in pet collars. While some of these products are efficacious, most, at best, offer protection of a very limited duration. Furthermore, many of the methods are often not successful in reducing insect populations on the pet for one or more of the following reasons: (1) failure of owner compliance (frequent administration is required); (2) behavioral or physiological intolerance of the pet to the pesticide product or means of administration; and (3) the emergence of insect populations resistant to the prescribed dose of pesticide.

An alternative method for controlling insect infestation is the use of insect vaccines to be administered to animals prior to or during insect infestation. However, despite considerable interest in developing anti-insect reagents, no insect vaccine presently exists of which the inventors are aware.

Calreticulins are calcium-binding proteins that have

been found in the endoplasmic reticulum of a number of mammals (e.g., humans, rabbits, rats, mice), endoparasites (e.g., *Schistosoma mansoni* and *Onchocerca volvulus*) and even in the barley plants. In contrast, the inventors are not aware of any reports in the literature identifying calreticulin as a component of insect saliva, and in fact, the only saliva in which calreticulin has been found is in tick saliva (Jaworski, Ph.D. Dissertation, Ohio State University, Volume 52/08-B of Dissertation Abstracts International, 1991 and Jaworski et al., *Biochem. Cell Biol.*, 71:11-12, 1993). That calreticulin is a component of insect saliva is unexpected even in view of the report of calreticulin in tick saliva not only because insects are phylogenetically different from ticks and other arachnids, but also because insects arose as a monophyletic order within the Class *Insecta* approximately 150 million years ago. Furthermore, insects in general, and fleas in particular, feed from their hosts in a manner quite different from ticks. For example, fleas feed frequently and quickly, usually over minutes, while ticks spend several days preparing a bite wound before beginning to ingest blood and will feed uninterrupted for 5-7 days. Thus, the biological activity of components of flea saliva would be expected to differ from components of tick saliva. Indeed, studies by Jaworski, 1991, *ibid.*, indicate that administration of calreticulin to a tick host causes necrosis and hemorrhaging at the site of tick feeding.

In summary, there remains a need to develop a reagent

and a method to protect animals from insect infestation and diseases caused by such infestation.

SUMMARY OF THE INVENTION

The present invention relates to, in one embodiment, an isolated hematophagous insect calreticulin protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a flea calreticulin gene. A preferred flea calreticulin gene comprises nucleic acid sequences SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16; as such, a preferred flea calreticulin gene encodes a protein comprising amino acid sequences SEQ ID NO:2 and SEQ ID NO:12. Preferably, the insect calreticulin is derived from insects including fleas, midges, mosquitos, sand flies, black flies, horse flies, horn flies, deer flies, tsetse flies, buffalo flies, blow flies, stable flies, myiasis-causing flies, biting gnats, lice and true bugs, more preferably fleas of a genus including *Ctenocephalides*, *Cyopsyllus*, *Diamanus*, *Echidnophaga*, *Nosopsyllus*, *Pulex*, *Tunga*, *Oropsylla*, *Orchopeus* or *Xenopsylla*, and even more preferably a species including *Ctenocephalides felis*, *Ctenocephalides canis*, *Pulex irritans*, *Oropsylla (Thrassis) bacchi*, *Oropsylla (Diamanus) montana*, *Orchopeus howardi*, *Xenopsylla cheopis* or *Pulex simulans*.

Another embodiment of the present invention includes an isolated hematophagous insect nucleic acid molecule that hybridizes under stringent hybridization conditions to a flea calreticulin gene. In particular, the nucleic acid

molecule comprises a nucleic acid sequence having at least about 80% identity with nucleic acid sequence SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, and/or SEQ ID NO:16. Also included in the present invention are recombinant molecules and recombinant cells having a nucleic acid molecule of the present invention.

Yet another embodiment of the present invention includes a therapeutic composition for protecting an animal from hematophagous insect infestation, the composition comprising compounds including an isolated hematophagous insect calreticulin protein or a mimetope thereof, an isolated nucleic acid molecule capable of hybridizing under stringent hybridization conditions with a flea calreticulin gene, an isolated anti-hematophagous insect calreticulin antibody or a mimetope thereof, and/or a calreticulin inhibitory compound. The composition, when administered to an animal, is able to reduce hematophagous insect burden on the animal and in the environment of the animal. Preferred animals to protect with a therapeutic composition of the present invention include mammals and birds, with cats, dogs, sheep, cows, pigs, horses and goats being more preferred. The present invention also relates to a method to protect an animal from insect infestation, comprising treating an animal with a therapeutic composition of the present invention.

One aspect of the present invention includes a method to produce a hematophagous insect calreticulin protein comprising culturing in an effective medium a recombinant

cell transformed with a nucleic acid molecule encoding the protein to produce the protein.

Another aspect of the present invention includes a method to identify a compound capable of inhibiting hematophagous insect calreticulin activity, said method comprising: (a) contacting an isolated hematophagous insect calreticulin protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has calreticulin activity; and (b) determining if the putative inhibitory compound inhibits said calreticulin activity. The present invention also includes test kits to identify inhibitory compounds and inhibitory compounds identified using a test kit and/or method of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes hematophagous insect calreticulin nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, inhibitors of such proteins and the use of therapeutic compositions comprising such nucleic acid molecules, proteins, antibodies and/or inhibitors (i.e., inhibitory compounds) to protect an animal from insect infestation and insect allergic dermatitis. The present invention is particularly advantageous in that, since calreticulin can be found in the saliva of insects, therapeutic compositions of the present invention can be used to alter the ability of hematophagous insects to acquire and digest blood meals from an animal treated with

the composition. A primary function of calreticulin is to regulate calcium concentrations, such as calcium concentrations that regulate calcium-dependent blood coagulation. Without being bound by theory, it is believed that calreticulin is produced and secreted by the salivary gland of an insect as the insect feeds from a host and is washed into the gut with the blood meal. During blood meal acquisition, therefore, the secreted calreticulin is believed to influence blood coagulation and blood vessel dilation at the feeding site by, for example, interfering with platelet aggregation and/or altering nitric oxide synthetase production. During blood meal digestion in the gut, calreticulin can also reduce blood clotting by, for example modifying the activity of components (e.g., blood factors) of the blood coagulation pathways.

One embodiment of the present invention is an isolated hematophagous insect calreticulin protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a flea calreticulin gene. According to the present invention, hematophagous insects are external living insects that attach and feed through the skin of a host animal. Hematophagous insects can live on a host animal or attach temporarily to an animal in order to feed.

As used herein, a flea calreticulin gene includes all nucleic acid sequences related to a natural flea calreticulin gene such as regulatory regions that control production of a flea calreticulin protein encoded by that

gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. A flea calreticulin gene can be an isolated natural flea calreticulin gene or a homologue thereof. A flea calreticulin gene can be included in a nucleic acid molecule encoding a hybrid protein, a fusion protein, a multivalent protein or a truncation fragment. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules are used to identify similar nucleic acid molecules. Additional definition and examples of such conditions are provided herein.

A preferred flea calreticulin gene of the present invention encodes a protein that includes, but is not limited to, the amino acid sequence represented herein as SEQ ID NO:2. Also preferred is a flea calreticulin gene encoding a protein having the amino acid sequence SEQ ID NO:12, which apparently represents the full-length protein. A more preferred flea calreticulin gene of the present invention includes, but is not limited to, the nucleic acid sequence referred to herein as SEQ ID NO:1 and/or the following nucleic acid sequences: SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, and/or SEQ ID NO:16. The determination of SEQ ID NO:1 and SEQ ID NO:2, as well as other sequences presented herein, is described in the Examples. The included SEQ ID NO's represent nucleic acid and amino acid sequences deduced according to methods disclosed in the Examples. It should be not d that since

nucleic acid and amino acid sequencing technology is not entirely error-free, the fore-going SEQ ID NO's, at best, represent an apparent nucleic acid sequence of the isolated nucleic acid molecule used to obtain SEQ ID NO:1 or an amino acid sequence deduced from SEQ ID NO:1 (i.e., SEQ ID NO:2), or other sequences presented herein.

Suitable flea calreticulin genes of the present invention encode calreticulin proteins that can be isolated from flea saliva. Flea saliva refers to the material released from the mouth of a flea when the flea attempts to feed in response to a temperature differential between the skin temperature of a host animal and the temperature of the air surrounding the host, or in response to an artificially created temperature differential such as exists in an artificial flea feeding apparatus.

According to the present invention, an isolated hematophagous insect calreticulin protein is a hematophagous insect calreticulin protein that has been removed from its natural milieu. An isolated hematophagous insect calreticulin protein can, for example, be obtained from its natural source, be produced using recombinant DNA technology, or be synthesized chemically. As used herein, an isolated hematophagous insect calreticulin protein can be a full-length protein or a homologue thereof.

In one embodiment, an isolated hematophagous insect calreticulin protein of the present invention is a protein isolated from the saliva of an insect. As noted above, such an "isolated" protein can be a natural protein, a

synthetic protein, or a recombinant protein.

A preferred isolated hematophagous insect calreticulin protein of the present invention is a protein that, when administered to an animal, is capable of protecting that animal from insect infestation and/or allergic dermatitis. As used herein, the phrase "to protect an animal from insect infestation" refers to reducing the potential for insect population expansion on and around an animal (i.e., reducing the insect burden). At any given time, a certain percentage of an insect population can be on a host animal whereas the remainder can be in the environment surrounding the animal (i.e., in the environment of the animal). A host animal, as used herein, is an animal from which insects can feed. The environment can be of any size such that insects in the environment are able to attach onto and detach from a host animal. Preferably, the insect population size is decreased, optimally to an extent that the animal is no longer bothered by such insects. As such, it is desirable not only to reduce the insect burden on an animal per se, but also to reduce the insect burden in the environment surrounding the animal.

A preferred hematophagous insect calreticulin protein of the present invention can protect an animal from insect infestation by reducing calreticulin activity at the feeding site or in an insect (e.g., in the midgut) feeding from an animal administered such a protein. Such a reduction in calreticulin activity can be the result of the ability of the protein to reduce calcium binding activity

and/or other activities of the insect calreticulin by, for example, eliciting an immune response against the calreticulin in the insect feeding from the animal. As such, a preferred hematophagous insect calreticulin protein of the present invention can include at least one epitope capable of eliciting production of an antibody capable of binding to an insect calreticulin protein. It is to be noted that other calreticulin activities include but are not limited to, (a) ability to bind to other factors such as magnesium, cellular integrins, extracellular matrix proteins, DNA domains of nuclear hormone receptors, and/or resident endoplasmic reticulum proteins; (b) ability to affect protein translocation from an endoplasmic reticulum; and/or (c) ability to act as an agonist or antagonist of other calcium binding proteins.

Administration of an isolated hematophagous insect calreticulin protein of the present invention can (a) alter the ability of a hematophagous insect to feed from an animal (e.g., by altering the volume per feeding or altering the time and frequency of feeding) as a result of interfering with platelet aggregation or vasodilation; (b) interfere with blood coagulation at the feeding site or in the midgut of an insect that feeds from an animal; (c) interfere with the activation of blood complement ingested from an animal in the midgut of an insect; (d) reduce the number of eggs produced and/or their subsequent viability; and/or (e) modulate the immune system of an animal by regulating, for example, the activation of cells involved

in an immune response, chemotaxis, lymphocyte responsiveness, and cytokine production. As used herein, the ability of a hematophagous insect calreticulin protein to alter insect feeding behavior refers to the protein's ability to alter blood meal acquisition and/or blood meal digestion.

In one embodiment, a hematophagous insect calreticulin protein of the present invention includes a protein that, when administered to an animal, is capable of substantially desensitizing the animal to allergic dermatitis. Preferred types of allergic dermatitis to desensitize an animal against include flea allergic dermatitis, *Culicoides* allergic dermatitis and mosquito allergic dermatitis, with flea allergic dermatitis being more preferred. As used herein, "desensitizing an animal" refers to treating an animal in such a manner that the animal does not exhibit a hypersensitive response to an allergen. Hypersensitivity refers to a state of altered reactivity in which an animal, having been previously exposed to a compound, exhibits an allergic response to the compound upon subsequent exposures. The term "allergen" primarily refers to foreign compounds capable of causing an allergic response. The term can be used interchangeably with the term "antigen," especially with respect to a foreign compound capable of inducing symptoms of immediate and/or delayed hypersensitivity. Preferably, an animal is substantially desensitized to allergic dermatitis when the animal is blocked from having a hypersensitive response (e.g., edema,

erythema, itching, inflammation) to the bite of an insect.

The ability of a hematophagous insect calreticulin protein to protect an animal from infestation or allergic dermatitis, can be tested using techniques known to those skilled in the art. Methods to measure insect infestation include determining insect viability, fecundity of female insects, reproductive capacity of male insects, viability of insect eggs, blood feeding behavior, viability of insect larvae, and/or development and maturation of larvae into adults. Techniques to test allergic dermatitis include standard allergen skin tests (e.g., IDST), immunoabsorbent assays (e.g., ELISA, Western blot, radioimmunoprecipitation assay), and passive cutaneous anaphylaxis.

As heretofore stated, a hematophagous insect calreticulin protein of the present invention can be a full-length natural protein or a homologue thereof. As used herein, a homologue can be a hematophagous insect calreticulin protein in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycosylphosphatidyl inositol). A homologue of a hematophagous insect calreticulin protein is a protein having an amino acid sequence that is sufficiently similar to a natural hematophagous insect calreticulin protein amino acid sequence that a nucleic acid sequence encoding the

homologue is capable of hybridizing under stringent conditions to (i.e., with) a nucleic acid sequence encoding the natural hematophagous insect calreticulin protein amino acid sequence. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules, including oligonucleotides, are used to identify similar nucleic acid molecules. Such standard conditions are disclosed, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989. The minimal size of a protein homologue of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. As such, the size of the nucleic acid molecule encoding such a protein homologue is dependent on nucleic acid composition and percent homology between the nucleic acid molecule and complementary sequence as well as upon hybridization conditions per se (e.g., temperature, salt concentration, and formamide concentration). The minimal size of such nucleic acid molecules is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecules are GC-rich and at least about 15 to about 17 bases in length if they are AT-rich. As such, the minimal size of a nucleic acid molecule used to encode a hematophagous insect calreticulin protein homologue of the present invention is from about 12 to about 18 nucleotides in length. There is no limit, other than a practical

limit, on the maximal size of such a nucleic acid molecule in that the nucleic acid molecule can include a portion of a gene, an entire gene, or multiple genes, or portions thereof. Similarly, the minimal size of a hematophagous insect calreticulin protein homologue of the present invention is from about 4 to about 6 amino acids in length, but can be of any longer length depending on whether a full-length, multivalent (i.e., protein having more than one domain each of which has a protective function), fusion, hybrid or functional portions of such proteins are desired.

Hematophagous insect calreticulin protein homologues can be the result of allelic variation of a natural gene encoding a hematophagous insect calreticulin protein. A natural gene refers to the form of the gene found most often in nature. Hematophagous insect calreticulin protein homologues can be produced using techniques known in the art including, but not limited to, direct modifications to a gene encoding a protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

Preferred hematophagous insect calreticulin protein homologues of the present invention are capable of protecting an animal from hematophagous insect infestation or allergic dermatitis resulting from the bites of insects in a similar manner as the natural hematophagous insect calreticulin protein counterpart. The ability of a hematophagous insect calreticulin protein homologue to

protect an animal from hematophagous insect infestation or allergic dermatitis can be tested by the methods disclosed herein.

A preferred hematophagous insect calreticulin protein of the present invention comprises (i.e., includes, but is not limited to) an amino acid sequence that is at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to an amino acid sequence represented by SEQ ID NO:2 and/or to amino acid sequences spanning amino acids from about 10 through about 360 of SEQ ID NO:12. A more preferred hematophagous insect calreticulin protein of the present invention comprises an amino acid sequence encoded by a nucleic acid molecule that is at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to a nucleic acid sequence represented by SEQ ID NO:1, and/or to a nucleic acid sequence spanning about nucleotide 360 through about 860 of SEQ ID NO:11. For example, Meinkoth et al, 1984, *Anal. Biochem 138*, 267-284, provide formulae to calculate the appropriate hybridization and wash conditions to achieve hybridization at various degrees of nucleic acid identity as disclosed herein. Meinkoth et al., *ibid.*, is incorporated by reference herein in its entirety.

In a preferred embodiment, a hematophagous insect calreticulin protein of the present invention comprises an amino acid sequence represented by SEQ ID NO:2 and/or SEQ

ID NO:12. In particular, a hematophagous insect calreticulin protein of the present invention comprises an amino acid sequence encoded by a nucleic acid sequence represented by SEQ ID NO:1, SEQ ID NO:11 and/or SEQ ID NO:14. A preferred embodiment includes flea calreticulin proteins $PCtCal_{85}$, $PHIS\text{-}PCtCal_{257}$, $PCtCal_{403}$ and $PHIS\text{-}PCtCal_{403}$, the production of which is described in the Examples.

As will be apparent to one of skill in the art, the present invention is intended to apply to all hematophagous insects. A preferred insect of the present invention from which to obtain hematophagous insect calreticulin proteins, and/or from which to identify proteins that can then be produced recombinantly or synthetically, include fleas; flies, such as midges (e.g., *Culicoides*), mosquitos, sand flies, black flies, horse flies, horn flies (e.g., *Haematobia irritans irritans*), deer flies, tsetse flies, buffalo flies, blow flies, stable flies, myiasis-causing flies and biting gnats; lice and true bugs (e.g., *Rhodinus* and *Cimex*), such as bed bugs and kissing bugs, including those carrying Chagas disease. A more preferred insect includes a flea, a myiasis-causing fly and a horn fly. A preferred flea of the present invention includes a flea being of the genus *Ctenocephalides*, *Cyopsyllus*, *Diamanus*, *Echidnophaga*, *Nosopsyllus*, *Pulex*, *Tunga*, *Oropsylla*, *Orchopeus* or *Xenopsylla*. A more preferred flea of the present invention includes a flea being of the species *Ctenocephalides felis*, *Ctenocephalides canis*, *Pulex irritans*, *Oropsylla (Thrassis) bacchi*, *Oropsylla (Diamanus)*

montana, *Orchopeus howardi*, *Xenopsylla cheopis* or *Pulex simulans*.

Another embodiment of the present invention is an isolated hematophagous insect calreticulin nucleic acid molecule that hybridizes under stringent hybridization conditions to a flea calreticulin gene. In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to human manipulation). As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated nucleic acid molecule can include DNA, RNA, or derivatives of either DNA or RNA.

An isolated nucleic acid molecule of the present invention can be obtained from its natural source either as an entire (i.e., complete) gene or a portion thereof capable of forming a stable hybrid with that gene. As used herein, the phrase "at least a portion of" an entity refers to an amount of the entity that is at least sufficient to have the functional aspects of that entity. For example, at least a portion of a nucleic acid sequence, as used herein, is an amount of a nucleic acid sequence capable of forming a stable hybrid with the corresponding gene under stringent hybridization conditions. An isolated nucleic acid molecule of the present invention can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated hematophagous insect calreticulin

nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants (the definition of which is known to those skilled in the art) and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications do not substantially interfere with the nucleic acid molecule's ability to encode a hematophagous insect calreticulin protein of the present invention or to form stable hybrids under stringent conditions with natural nucleic acid molecule isolates.

An isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one hematophagous insect calreticulin protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a hematophagous insect calreticulin protein. As heretofore disclosed, a hematophagous insect calreticulin protein of the present invention include, but are not limited to, proteins having full-length hematophagous insect calreticulin protein coding regions and homologues thereof.

As heretofore disclosed, a hematophagous insect

calreticulin gene includes all nucleic acid sequences related to a natural hematophagous insect calreticulin gene such as regulatory regions that control production of a hematophagous insect calreticulin protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. A nucleic acid molecule of the present invention can be an isolated natural hematophagous insect calreticulin nucleic acid molecule or a homologue thereof. A nucleic acid molecule of the present invention can include one or more regulatory regions, full-length or partial coding regions, or combinations thereof. The minimal size of a hematophagous insect calreticulin nucleic acid molecule of the present invention is the minimal size capable of forming a stable hybrid under stringent hybridization conditions with a corresponding natural gene.

A hematophagous insect calreticulin nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook et al., *ibid.*). For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, polymerase chain reaction (PCR) amplification and/or mutagenesis of selected regions of a

nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologues can be selected from a mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid (e.g., the ability of a homologue to protect an animal from insect infestation or from allergic dermatitis) and/or by hybridization with an isolated hematophagous insect calreticulin gene under stringent conditions.

A preferred nucleic acid molecule of the present invention includes a nucleic acid molecule that encodes an amino acid sequence having at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identity to amino acid sequence represented as SEQ ID NO:2, or to amino acid sequences spanning amino acids from about 10 through about 360 of SEQ ID NO:12. A more preferred nucleic acid molecule comprises a nucleic acid sequence having at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identity to a nucleic acid sequence represented as SEQ ID NO:1 or to a nucleic acid sequence spanning about nucleotide 360 through about 860 of SEQ ID NO:11, or to the complements of those sequences.

A particularly preferred nucleic acid molecule of the present invention includes a nucleic acid sequence encoding an amino acid sequence represented herein as SEQ ID:2

and/or SEQ ID NO:12; and/or a nucleic acid molecule that includes a nucleic acid sequence represented herein as SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, and/or SEQ ID NO:16. One preferred embodiment includes nucleic acid molecules $nCtCal_{257}$, $nCtCal_{850}$, $nCtCal_{550}$, $nCtCal_{556}$, $nCtCal_{1589}$, $nCtCal_{1209}$, $nCtCal_{1212}$, $nCtCal_{665}$, $nCtCal_{750}$, and $nCtCal_{1218}$.

Knowing a nucleic acid molecule of a hematophagous insect calreticulin protein of the present invention allows one skilled in the art to make copies of that nucleic acid molecule as well as to obtain a nucleic acid molecule including additional portions of hematophagous insect calreticulin protein-encoding genes (e.g., nucleic acid molecules that include a full-length coding region and/or transcription and/or translation control regions), and/or hematophagous insect calreticulin nucleic acid molecule homologues. Knowing a portion of an amino acid sequence of a hematophagous insect calreticulin protein of the present invention allows one skilled in the art to clone nucleic acid sequences encoding such a hematophagous insect calreticulin protein. Additional desired hematophagous insect calreticulin nucleic acid molecules can be obtained in a variety of ways including, but not limited to, screening appropriate expression libraries with antibodies which bind to a hematophagous insect calreticulin protein of the present invention; use of oligonucleotide probes of the present invention to screen appropriate libraries or DNA; and PCR amplification of appropriate libraries, or RNA

or DNA using oligonucleotide primers of the present invention (genomic and/or cDNA libraries can be used). To isolate a hematophagous insect calreticulin nucleic acid molecule, preferred cDNA libraries include cDNA libraries made from insect salivary glands. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid*. The Examples section includes examples of the isolation of a cDNA sequence encoding a hematophagous insect calreticulin protein of the present invention.

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as a flea calreticulin gene. A preferred oligonucleotide is capable of hybridizing, under stringent conditions, with a nucleic acid molecule that encodes a protein comprising SEQ ID NO:2 and/or SEQ ID NO:12, or complements thereof. A more preferred oligonucleotide is capable of hybridizing, under stringent conditions, to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:11, and/or SEQ ID NO:14, or to complements thereof (i.e., SEQ ID NO:16, SEQ ID NO:13, and/or SEQ ID NO:15, respectively).

Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimal size of such oligonucleotides is the size required to form a stable hybrid between a given oligonucleotide and the complementary sequence on another nucleic acid molecule of

the present invention. Minimal size characteristics are disclosed herein. The size of the oligonucleotide must also be sufficient for the use of the oligonucleotide in accordance with the present invention. Oligonucleotides of the present invention can be used in a variety of applications including, but not limited to, as probes to identify additional nucleic acid molecules, as primers to amplify or extend nucleic acid molecules or in therapeutic applications to inhibit, for example, expression of calreticulin proteins by insects. Such therapeutic applications include the use of such oligonucleotides in, for example, antisense-, triplex formation-, ribozyme- and/or RNA drug-based technologies. The present invention, therefore, includes such oligonucleotides and methods to interfere with the production of hematophagous insect calreticulin proteins by use of one or more of such technologies.

The present invention also includes a recombinant vector, which includes a hematophagous insect calreticulin nucleic acid molecule of the present invention inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to a hematophagous insect calreticulin nucleic acid molecule of the present invention. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the

cloning, sequencing, and/or otherwise manipulating of a hematophagous insect calreticulin nucleic acid molecule of the present invention. One type of recombinant vector, herein referred to as a recombinant molecule and described in more detail below, can be used in the expression of nucleic acid molecules of the present invention. Preferred recombinant vectors are capable of replicating in the transformed cell. Preferred nucleic acid molecules to include in recombinant vectors of the present invention are disclosed herein.

As heretofore disclosed, one embodiment of the present invention is a method to produce a hematophagous insect calreticulin protein of the present invention by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell that is capable of expressing the hematophagous insect calreticulin protein, the recombinant cell being produced by transforming a host cell with one or more nucleic acid molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention

can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a host cell are disclosed herein.

Suitable host cells to transform include any cell that can be transformed and that can express the introduced hematophagous insect calreticulin protein. Such cells are, therefore, capable of producing a hematophagous insect calreticulin protein of the present invention after being transformed with at least one nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule. Suitable host cells of the present invention can include bacterial, fungal (including yeast), insect, animal and plant cells. Preferred host cells include bacterial, yeast, insect and mammalian cells, with bacterial (e.g., *E. coli*) and insect (e.g., *Spodoptera*) cells being particularly preferred.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used

herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, insect, animal, and/or plant cells. As such, nucleic acid molecules of the present invention can be operatively linked to expression vectors containing regulatory sequences such as promoters, operators, repressors, enhancers, termination sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention. As used herein, a transcription control sequence includes a sequence which is capable of controlling the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art.

Preferred transcription control sequences include those which function in bacterial, yeast, helminth, insect and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (such as lambda p_L and lambda p_R and fusions that include such promoters), bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoters (such as Sindbis virus subgenomic promoters), baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, poxvirus, adenovirus, simian virus 40, retrovirus actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with a DNA sequence encoding a hematophagous insect calreticulin protein.

Expression vectors of the present invention may also contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed hematophagous insect calreticulin protein to be secreted from the cell that

produces the protein. Suitable signal segments include a hematophagous insect calreticulin protein signal segment or any heterologous signal segment capable of directing the secretion of a hematophagous insect calreticulin protein, including fusion proteins, of the present invention. Preferred signal segments include, but are not limited to, flea calreticulin, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments.

Expression vectors of the present invention may also contain fusion sequences which lead to the expression of inserted nucleic acid molecules of the present invention as fusion proteins. Inclusion of a fusion sequence as part of a hematophagous insect calreticulin nucleic acid molecule of the present invention can enhance the stability during production, storage and/or use of the protein encoded by the nucleic acid molecule. Furthermore, a fusion segment can function as a tool to simplify purification of a hematophagous insect calreticulin protein, such as to enable purification of the resultant fusion protein using affinity chromatography. A suitable fusion segment can be a domain of any size that has the desired function (e.g., increased stability and/or purification tool). It is within the scope of the present invention to use one or more fusion segments. Fusion segments can be joined to amino and/or carboxyl termini of an insect calreticulin protein. Linkages between fusion segments and hematophagous insect calreticulin proteins can be

constructed to be susceptible to cleavage to enable straight-forward recovery of the hematophagous insect calreticulin proteins. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid sequence that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of a hematophagous insect calreticulin protein.

A recombinant molecule of the present invention is a molecule that can include at least one of any nucleic acid molecule heretofore described operatively linked to at least one of any transcription control sequence capable of effectively regulating expression of the nucleic acid molecule(s) in the cell to be transformed. A preferred recombinant molecule includes one or more nucleic acid molecules that encode one or more hematophagous insect calreticulin proteins, such as those disclosed herein.

It may be appreciated by one skilled in the art that use of recombinant DNA technologies can improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid

molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant protein production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing the resultant protein.

In accordance with the present invention, a recombinant cell can be used to produce a hematophagous insect calreticulin protein of the present invention by culturing such cell under conditions effective to produce such a protein, and recovering the protein. Effective conditions to produce a protein include, but are not limited to, appropriate media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An appropriate, or effective, medium refers to any medium in which a recombinant cell of the present invention, when cultured, is capable of producing a hematophagous insect calreticulin protein. Such a medium is typically an

aqueous medium comprising assimilable carbohydrate, nitrogen and phosphate sources, as well as appropriate salts, minerals, metals and other nutrients, such as vitamins. The medium may comprise complex nutrients or may be a defined minimal medium.

Cells of the present invention can be cultured in conventional fermentation bioreactors, which include, but are not limited to, batch, fed-batch, cell recycle, and continuous fermentors. Culturing can also be conducted in shake flasks, test tubes, microtiter dishes, and petri plates. Culturing is carried out at a temperature, pH and oxygen content appropriate for the recombinant cell. Such culturing conditions are well within the expertise of one of ordinary skill in the art.

Depending on the vector and host system used for production, resultant hematophagous insect calreticulin proteins may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane.

The present invention also includes isolated antibodies against hematophagous insect calreticulin proteins of the present invention (i.e., anti-hematophagous insect calreticulin protein antibodies) and their use to reduce insect infestation on a host animal as well as in the environment of the animal. An anti-hematophagous insect calreticulin protein antibody is an antibody capable

of selectively binding to a hematophagous insect calreticulin protein. An anti-hematophagous insect calreticulin protein antibody preferably binds to the hematophagous insect calreticulin protein in such a way as to reduce the activity of that protein.

Isolated antibodies are antibodies that have been removed from their natural milieu. The term "isolated" does not refer to the state of purity of such antibodies. As such, isolated antibodies can include anti-sera containing such antibodies, or antibodies that have been purified to varying degrees. As used herein, the term "selectively binds to" refers to the ability of such antibodies to preferentially bind to the hematophagous insect calreticulin protein against which the antibody was raised (i.e., to be able to distinguish that protein from unrelated components in a mixture.). Binding affinities typically range from about 10^3 M^{-1} to about 10^{12} M^{-1}. Binding can be measured using a variety of methods known to those skilled in the art including immunoblot assays, immunoprecipitation assays, radioimmunoassays, enzyme immunoassays (e.g., ELISA), immunofluorescent antibody assays and immunoelectron microscopy; see, for example, Sambrook et al., *ibid*.

Antibodies of the present invention can be either polyclonal or monoclonal antibodies. Antibodies of the present invention include functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies, that are capable of

selectively binding to at least one of the epitopes of the protein used to obtain the antibodies. Antibodies of the present invention also include chimeric antibodies that can bind to more than one epitope. Preferred antibodies are raised in response to proteins that are encoded, at least in part, by a hematophagous insect calreticulin nucleic acid molecule of the present invention.

Anti-hematophagous insect calreticulin protein antibodies of the present invention include antibodies raised in an animal administered a hematophagous insect calreticulin protein of the present invention that exert their effect when insects feed from the treated animal's blood containing such antibodies. Anti-hematophagous insect calreticulin protein antibodies of the present invention also include antibodies raised in an animal against one or more hematophagous insect calreticulin proteins of the present invention that are then recovered from the animal using techniques known to those skilled in the art. Yet additional antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed for hematophagous insect calreticulin protein of the present invention. Antibodies produced against defined proteins can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a therapeutic composition.

Anti-hematophagous insect calreticulin protein

antibodies of the present invention have a variety of uses that are within the scope of the present invention. For example, such antibodies can be used in a therapeutic composition of the present invention to passively immunize an animal in order to protect the animal from insect infestation. Anti-hematophagous insect calreticulin protein antibodies can also be used as tools to screen expression libraries and/or to recover desired proteins of the present invention from a mixture of proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to insects in order to kill insects. Targeting can be accomplished by conjugating (i.e., stably joining) such antibodies to cytotoxic agents using techniques known to those skilled in the art.

A preferred anti-hematophagous insect calreticulin protein antibody of the present invention can selectively bind to, and preferentially reduce calreticulin activity of, a flea calreticulin. A preferred anti-flea calreticulin antibody can selectively bind to a flea calreticulin comprising SEQ ID NO:2 and/or SEQ ID NO:12. A more preferred anti-flea calreticulin antibodies can selectively bind to a flea calreticulin encoded by a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:11 and/or SEQ ID NO:14.

The present invention also includes the use of active calreticulin proteins of the present invention to identify compounds that inhibit calreticulin activity (i.e.,

calreticulin inhibitory compounds), and preferably a calreticulin inhibitory compound that can be included in a therapeutic composition of the present invention to be administered to animals. A method to identify a compound capable of inhibiting hematophagous insect calreticulin activity, comprises: (1) contacting (e.g., combining, mixing) an isolated hematophagous insect calreticulin protein with a putative (i.e., candidate) inhibitory compound under conditions in which, in the absence of the compound, the protein has calreticulin activity; and (2) determining if the putative inhibitory compound inhibits the calreticulin activity. Putative inhibitory compounds to screen include natural or synthetic molecules, antibodies (including functional equivalents thereof) and substrate analogs. Methods to determine calreticulin activity are known to those skilled in the art. Examples of such methods include measuring the ability of the protein (a) to bind calcium, magnesium, cellular integrins, extracellular matrix proteins, DNA domains of a nuclear hormone receptor, and/or resident endoplasmic reticulum proteins; (b) to affect protein translocation from an endoplasmic reticulum; and/or (c) to act as an agonist or an antagonist of other calcium binding proteins (e.g., calmodulin, calnexin).

The present invention also includes a test kit to identify a compound capable of inhibiting hematophagous insect calreticulin activity. Such a test kit includes an isolated hematophagous insect calreticulin protein having

calreticulin activity and a means for determining the extent of inhibition of calreticulin activity in the presence of (i.e., effected by) a putative inhibitory compound. Examples of such means are well known to those skilled in the art, some of which are disclosed herein.

The present invention also includes hematophagous insect calreticulin inhibitory compounds isolated by such a method, and/or test kit, and their use to inhibit any hematophagous insect calreticulin that is susceptible to such an inhibitory compound.

It is to be appreciated that the present invention also includes mimetopes of compounds of the present invention that can be used in accordance with methods as disclosed for compounds of the present invention. As used herein, a mimetope of a proteinaceous compound of the present invention (e.g., a hematophagous insect calreticulin protein, an anti-calreticulin antibody, a proteinaceous inhibitor of hematophagous insect calreticulin activity) refers to any compound that is able to mimic the activity of that proteinaceous compound, often because the mimetope has a structure that mimics that of the proteinaceous compound. For example, a mimetope of a hematophagous insect calreticulin protein is a compound that has an activity similar to that of an isolated hematophagous insect calreticulin protein of the present invention. Mimetopes can be, but are not limited to: peptides that have been modified to decrease their susceptibility to degradation; anti-idiotypic and/or

catalytic antibodies, or fragments thereof; non-proteinaceous immunogenic portions of an isolated protein (e.g., carbohydrate structures); and synthetic or natural organic molecules, including nucleic acids and carbohydrates. Such mimetopes can be designed using computer-generated structures of proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner.

The present invention includes therapeutic compositions, also referred to herein as compositions, that include a (i.e., at least one) compound of the present invention. Preferred compounds to include in a composition of the present invention include an isolated hematophagous insect calreticulin protein or a mimetope thereof, an isolated nucleic acid molecule capable of hybridizing under stringent hybridization conditions with a flea calreticulin gene, an isolated anti-hematophagous insect calreticulin antibody or a mimetope thereof, and/or a calreticulin inhibitory compound, in which the composition, when administered to an animal, is able to reduce hematophagous insect burden on the treated animal and/or in the environment of the animal. Such a therapeutic composition can protect an animal from hematophagous insect infestation by reducing calreticulin activity in hematophagous insects feeding from the treated animal, thereby reducing

hematophagous insect burden on the animal and in the environment of the animal. Suitable and preferred hematophagous insects to target are disclosed herein. Particularly preferred hematophagous insects to target are fleas.

Compositions of the present invention can also include other components such as a pharmaceutically acceptable excipient, an adjuvant, and/or a carrier. For example, compositions of the present invention can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, m- or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water

or saline can be added prior to administration.

In one embodiment of the present invention, the composition can also include an immunopotentiator, such as an adjuvant or a carrier. Adjuvants are typically substances that generally enhance the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, Freund's adjuvant; other bacterial cell wall components; aluminum-based salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins; viral coat proteins; other bacterial-derived preparations; gamma interferon; block copolymer adjuvants, such as Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, GA); Ribi adjuvants (available from Ribi ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives, such as Quil A (available from Superfos Biosector A/S, Denmark). Carriers are typically compounds that increase the half-life of a therapeutic composition in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release formulations, biodegradable implants, liposomes, bacteria, viruses, oils, esters, and glycols. One embodiment is an encapsulated therapeutic composition that can be delivered orally.

One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release

vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ*. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

A preferred controlled release formulation of the present invention is capable of releasing a composition of the present invention into the blood of the treated animal at a constant rate sufficient to attain therapeutic dose levels of the composition to reduce calreticulin activity in insects feeding from the animal over a period of time ranging from about 1 to about 12 months. A controlled release formulation of the present invention is capable of effecting a treatment for preferably at least about 1 month, more preferably at least about 3 months and even more preferably for at least about 6 months, even more preferably for at least about 9 months, and even more preferably for at least about 12 months.

In order to protect an animal from hematophagous insect infestation, a therapeutic composition of the present invention is administered to the animal in an effective manner such that the calreticulin activity of hematophagous insects feeding from the blood stream of animals treated with the composition is reduced. As such,

a treated animal is an animal that is competent to reduce hematophagous insect burden by reducing calreticulin activity in insects feeding on that animal. Preferably, the calreticulin activity in the feeding insect is reduced by at least about 50 percent, more preferably by at least about 75 percent, and even more preferably by at least about 100 percent. Methods to administer compositions to the animal in order to render the animal competent depend on the nature of the composition and administration regime.

Animals administered a composition usually become competent, for example, about 3 to 6 weeks after a primary dose and within another about 2 to 4 weeks with at least one booster shot. Animals administered a composition including an anti-hematophagous insect calreticulin antibody or calreticulin inhibitory compound become competent as soon as appropriate serum levels of the antibody or inhibitory compound are achieved, usually within about one to about seven days. Methods to measure animal competency (i.e., ability of blood taken up by an insect feeding from the treated animal to reduce calreticulin activity in the insect) are known to those skilled in the art.

In accordance with the present invention, compositions are administered to an animal in a manner such that the animal becomes competent to reduce hematophagous insect calreticulin activity in a hematophagous insect that feeds from the competent animal. For example, a hematophagous insect calreticulin protein of the present invention, when

administered to an animal in an effective manner (i.e., using an appropriate protocol), is able to elicit (i.e., stimulate) an immune response that produces an antibody titer in the blood stream of the animal sufficient to reduce hematophagous insect calreticulin activity. Similarly, an anti-hematophagous insect calreticulin antibody of the present invention, when administered to an animal in an effective manner, is administered according to a protocol so as to be present in the animal's blood stream at a titer that is sufficient to reduce hematophagous insect calreticulin activity. A calreticulin inhibitory compound of the present invention, when administered to an animal in an effective manner, is administered according to a protocol so as to be present in the animal's blood stream at a concentration that is sufficient to reduce hematophagous insect calreticulin activity. A nucleic acid molecule of the present invention, such as an oligonucleotide or a nucleic acid molecule that encodes a calreticulin protein of the present invention, can also be administered in an effective manner, thereby reducing hematophagous insect calreticulin activity.

Compositions of the present invention can be administered to animals prior to or during hematophagous insect infestation. As noted above, when a hematophagous insect calreticulin protein of the present invention is administered to an animal, a time period is required for the animal to elicit an immune response before the animal is competent to inhibit calreticulin activity of

hematophagous insects feeding from that animal. Methods to obtain an immune response in an animal are known to those skilled in the art.

Acceptable protocols to administer compositions in an effective manner include individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. A suitable single dose is a dose that is capable of protecting an animal from hematophagous insect infestation when administered one or more times over a suitable time period. For example, a preferred single dose of calreticulin protein or a mimetope thereof ranges from about 1 microgram (μg) to about 10 milligrams (mg) per kilogram (kg) body weight of the animal. Booster vaccinations can be administered from about 2 weeks to several years after the original administration. Booster immunizations preferably are administered when the immune response of the animal becomes insufficient to protect the animal from hematophagous insect infestation. A preferred administration schedule is one in which from about 1 μg to about 10 mg of hematophagous insect calreticulin protein per kg body weight of the animal is administered from about one to about two times over a time period of from about 2 weeks to about 12 months. Modes of administration can include, but are not limited to, oral, nasal, topical, transdermal, rectal, and parenteral routes. Parenteral routes can include, but are not limited to subcutaneous,

intradermal, intravenous, and intramuscular routes.

In another embodiment, an anti-hematophagous insect calreticulin antibody composition or a mimetope thereof can be administered in an amount effective to induce a therapeutic effect such as can be determined by one skilled in the art. Anti-hematophagous insect calreticulin antibodies can be re-administered from about 1 hour to about biweekly for several weeks following the original administration. Booster treatments preferably are administered when the titer of antibodies of the animal becomes insufficient to protect the animal from hematophagous insect infestation. Suitable modes of administration are as disclosed herein and are known to those skilled in the art.

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a protective protein (e.g., hematophagous insect calreticulin protein, anti-hematophagous insect calreticulin antibody, or proteinaceous calreticulin inhibitory compound) or protective RNA (e.g., antisense RNA, ribozyme or RNA drug) in the animal to be protected from disease. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) direct injection (e.g., as "naked" DNA or RNA molecules, such as is taught, for example in Wolff et al., 1990, *Science 247*, 1465-1468) or (b) packaged as a recombinant virus particle vaccine or as a recombinant cell

vaccine (i.e., delivered to a cell by a vehicle selected from the group consisting of a recombinant virus particle vaccine and a recombinant cell vaccine).

A recombinant virus particle vaccine of the present invention includes a recombinant molecule of the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule is packaging-deficient. A number of recombinant virus particles can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, and retroviruses.

When administered to an animal, a recombinant virus particle vaccine of the present invention infects cells within the immunized animal and directs the production of a protective protein or RNA nucleic acid molecule that is capable of protecting the animal from disease caused by the bites of insects of the present invention. A preferred single dose of a recombinant virus particle vaccine of the present invention is from about 1×10^4 to about 1×10^7 virus plaque forming units (pfu) per kilogram body weight of the animal. Administration protocols are similar to those described herein for protein-based vaccines.

A recombinant cell vaccine of the present invention includes recombinant cells of the present invention that express at least one protein of the present invention. Preferred recombinant cells for use in this embodiment include *Salmonella*, *E. coli*, *Mycobacterium*, *S. frugiperda*, baby hamster kidney, myoblast G8, COS, MDCK and CRFK

recombinant cells, with *Salmonella* recombinant cells being more preferred. Such recombinant cells can be administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about 10^8 to about 10^{12} bacteria per kilogram body weight. Administration protocols are similar to those described herein for proteinaceous compositions of present invention. Recombinant cell vaccines can comprise whole cells or cell lysates.

Compositions of the present invention can be administered to any animal susceptible to hematophagous insect infestation, including warm-blooded animals. Preferred animals to treat include mammals and birds, with cats, dogs, humans, cattle, chinchillas, ferrets, goats, mice, minks, rabbits, raccoons, rats, sheep, squirrels, swine, chickens, ostriches, quail and turkeys as well as other furry animals, pets and/or economic food animals, being more preferred. Particularly preferred animals to protect are cats, dogs, sheep, cows, pigs, horses and goats.

One embodiment of the present invention is a method to protect an animal from insect infestation, comprising treating an animal with a composition of the present invention. It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a compound refers to one or more compounds. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. Thus, a composition of the

present invention can include one or more compounds that reduce the activity of hematophagous insect calreticulins. Suitable and preferred therapeutic compositions are disclosed herein.

Another embodiment of the present invention includes a method to desensitize a host animal to allergic dermatitis, comprising administering to the animal a hematophagous insect calreticulin protein of the present invention, or a nucleic acid molecule encoding such a protein. Suitable and preferred proteins and nucleic acid molecules are as disclosed for therapeutic compositions useful in reducing hematophagous insect burden in animals. Methods to administer such compounds are as disclosed in U.S. Patent Application Serial No. 08/319,590 (also referred to herein as S/N 08/318,590), *ibid.*

A hematophagous insect calreticulin protein of the present invention can be used in conjunction with other compounds capable of modifying an animal's hypersensitivity to insect bites. Examples of suitable hematophagous insect saliva proteins to utilize are disclosed in S/N 08/318,590, *ibid.* For example, in order to treat flea allergic dermatitis, an animal can be administered a flea calreticulin protein of the present invention in combination with one or more flea saliva proteins disclosed in S/N 08/318,590, *ibid.*, including FS-1 flea saliva extract, FS-2 flea saliva extract, and/or proteins included in those extracts. FS-1 and FS-2 flea saliva extracts include mixtures of flea saliva proteins that are collected

by the method described in detail in S/N 08/319,590, *ibid.* Preferred flea saliva proteins for use with a flea insect calreticulin protein of the present invention to treat flea allergic dermatitis include at least a portion of one or more of the following flea saliva proteins fspA, fspB, fspC1, fspC2, fspD1, fspD2, fspE, fspF, fspG, fspH, fspI, fspJ1, fspJ2, fspK, fspL1, fspL2, fspM1, fspM2, fspN1, fspN2 and fspN3, as disclosed in S/N 08/319,590, *ibid.* As used herein and in S/N 08/319,590, *ibid.*, at least a portion of a saliva protein refers to a portion of a saliva protein encoded by a nucleic acid molecule that is capable of hybridizing, under stringent conditions, with a nucleic acid encoding a full-length saliva protein of the invention disclosed in S/N 08,319,590, *ibid.* More preferred flea saliva proteins for use with a flea calreticulin protein of the present invention include at least a portion of fspE, fspF, fspG, fspH, fspI, fspJ1, fspJ2, fspK, fspL1, fspL2, fspM1, fspM2, fspN1, fspN2 and/or fspN3, with fspG, fspH, fspM1, fspM2, fspN1, fspN2, fspN3, and/or proteins in FS-2 being even more preferred.

Other useful compounds to administer with a hematophagous insect calreticulin protein of the present invention in order to treat allergic dermatitis are compounds that are capable of modifying the function of a cell involved in a hypersensitive response, compounds that reduce allergic reactions, such as systemic agents or anti-inflammatory agents (e.g., anti-histamines, anti-steroid reagents, anti-inflammatory reagents and reagents that

drive immunoglobulin heavy chain class switching from IgE to IgG). Suitable compounds useful for modifying the function of a cell involved in a hypersensitive response include, but are not limited to, antihistamines, cromolyn sodium, theophylline, cyclosporin A, adrenalin, cortisone, compounds capable of regulating cellular signal transduction, compounds capable of regulating cyclic adenosine monophosphate (cAMP) activity, and compounds that block IgE activity, such as peptides from IgE or IgE specific Fc receptors, antibodies specific for peptides from IgE or IgE-specific Fc receptors, or antibodies capable of blocking binding of IgE to Fc receptors.

Also included in the present invention is the use of a hematophagous insect calreticulin protein of the present invention to identify animals susceptible to or having allergic dermatitis. As used herein, an animal that is susceptible to allergic dermatitis refers to an animal that is genetically pre-disposed to developing allergic dermatitis and/or to an animal that has been primed with an antigen in such a manner that re-exposure to the antigen results in symptoms of allergic dermatitis that can be perceived by, for example, observing the animal or measuring antibody production by the animal to the antigen. As such, animals susceptible to allergic dermatitis can include animals having sub-clinical allergic dermatitis. Sub-clinical allergic dermatitis refers to a condition in which allergy symptoms cannot be detected by simply observing an animal, but can be manifest by the presence of

anti-hematophagous insect calreticulin protein antibodies within an affected animal. Sub-clinical allergic dermatitis can be detected using *in vivo* or *in vitro* assays of the present invention as disclosed in S/N 08/319,590, *ibid.*). Reference to animals having allergic dermatitis includes animals that do display allergy symptoms that can be detected by simply observing an animal and/or by using *in vivo* or *in vitro* assays of the present invention.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

This example describes the cloning and sequencing of a flea calreticulin nucleic acid molecule of the present invention as well as the deduced amino acid sequence of a flea calreticulin protein of the present invention.

A flea calreticulin nucleic acid molecule of about 257 nucleotides, referred to herein as $nCtCal_{257}$, was PCR amplified from a flea (*Ctenocephalides felis*) salivary gland cDNA library that was prepared from RNA isolated from flea salivary glands using standard protocols as described in Sambrook et al., *ibid.* Primers used in the PCR amplification were degenerate oligonucleotides designed to correspond to conserved regions of the published sequences of human, mouse, rabbit, *Onchocerca volvulus* and *Schistosoma mansoni* calreticulin genes, and were as follows: a degenerate "sense" primer having SEQ ID NO:3,

namely 5'-AARCCNGARGAYTGGGAYAARCC-3' (R is a mixture of A and G; N is a mixture of G, A, T and C; Y is a mixture of C and T); and a degenerate "antisense" primer having SEQ ID NO:4, namely 5'-GTRTAYTCNGGRTTRTCDATYTC-3' (D is a mixture of G, A, and T).

Resulting PCR products were screened using a ^{32}P-labelled oligonucleotide probe having SEQ ID NO:5, namely 5'- AAYCCNGARGGNGARTGGAA-3', which corresponds to amino acid residues 251 to 258 of the known human calreticulin protein sequence in a standard Southern blot procedure. PCR products that hybridized to the probe were gel purified, cloned into the TA Vector® System (available from Invitrogen, San Diego, CA), and subjected to standard DNA sequencing techniques. An about 257 nucleotide sequence of $nCtCal_{257}$ was determined and is presented as SEQ ID NO:1 (coding strand) and SEQ ID NO:16 (complementary strand). SEQ ID NO:1 apparently encodes a protein of about 85 amino acids, denoted herein as $PCtCal_{85}$, the amino acid sequence of which is presented as SEQ ID NO:2.

Flea calreticulin nucleic acid sequence SEQ ID NO:1 and flea calreticulin amino acid sequence SEQ ID NO:2 were compared with calreticulin nucleic acid and amino acid sequences characterized from other organisms. SEQ ID NO:1 was found to be about 69% identical to the nucleic acid sequence of the corresponding region of the gene encoding rabbit calreticulin; about 70% identical to the nucleic acid sequence of the corresponding region of the gene encoding human calreticulin; about 71% identical to the

nucleic acid sequence of the corresponding region of the gene encoding *S. mansoni* calreticulin; about 74% identical to the nucleic acid sequences of the corresponding regions of the genes encoding mouse calreticulin and rat calreticulin; and about 75% identical to the nucleic acid sequences of the corresponding regions of the genes encoding bovine calreticulin and *O. volvulus* calreticulin. SEQ ID NO:2 was found to be about 64% identical to the corresponding region of *S. mansoni* calreticulin; about 72% identical to the corresponding region of *O. volvulus* calreticulin; about 81% identical to the corresponding regions of rabbit calreticulin and human calreticulin; and about 82% identical to the corresponding region of mouse calreticulin.

Example 2

This example describes the cloning of additional flea calreticulin nucleic acid molecules of the present invention.

A flea calreticulin nucleic acid molecule of about 850 nucleotides, referred to herein as $nCtCal_{850}$ and including the 5′ domain of the flea calreticulin gene, was PCR amplified from a flea salivary gland cDNA library as described in Example 1. The primers used to amplify $nCtCal_{850}$ were as follows: an M13 reverse vector primer having SEQ ID NO:6, namely 5′-GGAAACAGCTATGACCATG-3′; and an antisense primer having SEQ ID NO:7, namely 5′-TGAACCAGACACCTTTGTAGTCAGG-3′, which corresponds to nucleotides from about 205 through about 230 of SEQ ID

NO:1.

Resulting PCR products were screened using a ^{32}P-labelled oligonucleotide probe corresponding to internal flea calreticulin nucleic acid sequence in a standard Southern blot procedure. PCR products that hybridized to the probe were gel purified, were cloned into the TA Vector® System (available from Invitrogen, San Diego, CA), and were subjected to standard DNA sequencing techniques.

Flea calreticulin nucleic acid molecules of about 550 and of about 556 nucleotides, referred to herein as $nCtCal_{550}$ and $nCtCal_{556}$, respectively, (each including the 3′ domain of the flea calreticulin gene) were PCR amplified from a flea salivary gland cDNA library as described in Example 1. The primers used to amplify the nucleic acid molecules were as follows: an M13 universal vector primer having SEQ ID NO:8, namely 5′-GTAAAACGACGGCCAGT-3′; and a sense primer having SEQ ID NO:9, namely 5′-GGAAGATTGGGACAAGCCAGAAC-3′, which corresponds to nucleotides from about 57 through about 79 of SEQ ID NO:1; and another sense primer having SEQ ID NO:10, namely 5′-TTGGGACAAGCCAGAACACATTCC-3′, which corresponds to nucleotides from about 63 through about 86 of SEQ ID NO:1. Resulting PCR products were screened using a ^{32}P-labelled oligonucleotide probe having SEQ ID NO:7 in a standard Southern blot procedure. PCR products that hybridized to the probe were gel purified, cloned into the TA Vector® System (available from Invitrogen, San Diego, CA), and subjected to standard DNA sequencing techniques.

Example 3

This Example discloses the production of a recombinant cell of the present invention.

Recombinant molecule pHis-nCtCal$_{257}$, containing flea calreticulin nucleic acid molecule nCtCal$_{257}$ described in Example 1 operatively linked to *trc* transcription control sequences and to a fusion sequence encoding a poly-histidine segment comprising 6 histidines followed by the amino terminal segment of the bacteriophage S10 capsid protein gene, was produced in the following manner. Nucleic acid molecule nCtCal$_{257}$ was removed from the recombinant vector described in Example 1 by restriction endonuclease digestion, gel purified and subcloned into expression vector pTrcHisA (available from Invitrogen). The resulting recombinant molecule, denoted pHis-nCtCal$_{257}$, was transformed into *E. coli* to form recombinant cell *E. coli*:pHis-nCtCal$_{257}$.

Example 4

This Example discloses the production of a flea calreticulin protein of the present invention by a recombinant cell of the present invention.

Recombinant cell *E. coli*:pHis-nCtCal$_{257}$, produced as described in Example 3, is cultured in shake flasks containing an enriched bacterial growth medium containing about 0.1 mg/ml ampicillin at about 37°C. When the cells reach an OD_{600} of about 0.3, expression of nCtCal$_{257}$ is induced by addition of about 1 mM isopropyl-β-D-thiogalactoside (IPTG), and the cells cultured for about 3

hours at about 37°C. Protein production is monitored by SDS PAGE of recombinant cell lysates, followed by Coomassie blue staining, using standard techniques. Recombinant cell *E. coli*:pHis-nCtCal$_{257}$ produces a fusion protein, denoted herein as PHIS-PCtCal$_{257}$, that migrates with an apparent molecular weight of about 11.5 kD. Such a protein is not produced by cells transformed with the pTrcHisA plasmid lacking a flea nucleic acid molecule insert.

Immunoblot analysis of recombinant cell *E. coli*:pHis-nCtCal$_{257}$ lysates indicates that the about 11.5-kD protein binds to a T7 tag monoclonal antibody (available from Novagen, Inc., Madison, WI) directed against the fusion portion of the recombinant PHIS-PCtCal$_{257}$ fusion protein.

Example 5

This Example describes the cloning of additional flea calreticulin nucleic acid molecules of the present invention.

Based on nucleic acid sequence analysis of nCtCal$_{850}$ and nCtCal$_{556}$, produced as described in Example 2, two additional flea calreticulin gene-specific primers were designed to PCR amplify two additional flea calreticulin nucleic acid molecules from a flea salivary gland cDNA library. Primers used to amplify a flea calreticulin nucleic acid molecule of about 665 nucleotides, referred to herein as nCtCal$_{665}$ and including the 5' domain of the flea calreticulin gene, included: the M13 reverse vector primer having SEQ ID NO:6; and Cal 4R, a flea calreticulin anti-sense primer having SEQ ID NO:17, namely 5' ATTAGGGTCAGGAATAGTTGCACGCTC 3'.

Primers used to amplify a flea calreticulin nucleic acid molecule of about 750 nucleotides, referred to herein as $nCtCal_{750}$ and including the 3' domain of the flea calreticulin gene, included: the M13 universal vector primer having SEQ ID NO:8; and Cal 3F, a flea calreticulin sense primer having SEQ ID NO:18, namely 5' CATGTATACACTTTGGTTGTTAAGC 3'. Nucleic acid molecules $nCtCal_{665}$ and $nCtCal_{750}$ were gel purified, cloned into the TA Vector® System (available from Invitrogen), and subjected to standard DNA sequencing techniques.

Yet another flea calreticulin nucleic acid molecule was produced in the following manner. Flea calreticulin nucleic acid molecule $nCtCal_{1218}$, which includes the entire putative coding region of the flea calreticulin gene, was PCR amplified from a flea salivary gland cDNA library using the following primers: Cal 5F, a flea calreticulin sense primer having SEQ ID NO:19, namely 5' ATAAATATGAAAGCAATTTTGATAACA 3'; and Cal 3R, a flea calreticulin anti-sense primer having SEQ ID NO:20, namely 5' TCACAGTTCATCGTGCTCAGCATCGAGTGT 3'. The amplified PCR product was gel purified, cloned into the TA Vector® System (available from Invitrogen), and subjected to standard DNA sequencing techniques.

Nucleic acid sequence analysis of nucleic acid molecules $nCtCal_{665}$, $nCtCal_{750}$, and $nCtCal_{1218}$ led to the deduction of nucleic acid sequence SEQ ID NO:11 as well as of the complement of SEQ ID NO:11, namely SEQ ID NO:13. As used herein, nucleic acid molecule $nCtCal_{1589}$ includes SEQ ID

NO:11 and SEQ ID NO:13. Translation of SEQ ID NO:11 suggests that nucleic acid molecule $nCtCal_{1589}$ encodes a full-length flea calreticulin protein of about 403 amino acids, referred to herein as $PCtCal_{403}$, represented by SEQ ID NO:12, assuming an open reading frame having an initiation codon spanning from about nucleotide 151 through 153 and a termination codon spanning from about nucleotide 1360 through 1362 of SEQ ID NO:11. The coding region encoding $PCtCal_{403}$, without the stop codon, is represented by nucleic acid molecule $nCtCal_{1209}$, having the nucleic acid sequence represented by SEQ ID NO:14 (the coding strand) and SEQ ID NO:15 (the complementary strand). The deduced amino acid sequence of SEQ ID NO:12 suggests a protein having a molecular weight of about 46kD and an estimated pI of about 4.23.

Flea calreticulin nucleic acid sequence SEQ ID NO:11 and flea calreticulin amino acid sequence SEQ ID NO:12 were compared with calreticulin nucleic acid and amino acid sequences characterized from other organisms. Since there was very little nucleotide homology in either the 5' or 3' domains between genes of various species, those regions were not compared. SEQ ID NO:11, between about nucleotides 360 and 860, was found to be about 66% identical to the nucleic acid sequence of the corresponding region of the gene encoding bovine calreticulin; about 68% identical to the nucleic acid sequence of the corresponding region of the gene encoding *Drosophila* calreticulin; about 70% identical to the nucleic acid sequences of the

corresponding regions of the genes encoding mouse and rat calreticulins; and about 71% identical to the nucleic acid sequence of the corresponding region of the gene encoding human calreticulin. SEQ ID NO:12, between about amino acids 10 and 360 (a core region least influencedby length or 5' or 3' heterogeneity), was found to be about 68% identical to the corresponding region of bovine calreticulin; about 69% identical to the corresponding region of rat calreticulin; about 70% identical to the corresponding regions of mouse calreticulin and human calreticulin; and about 76% identical to the corresponding region of *Drosophila* calreticulin.

Example 6

This Example discloses the production of another recombinant cell of the present invention.

Recombinant molecule pTrpHis-nCtCal$_{1212}$, containing a flea calreticulin nucleic acid molecule comprising the entire putative coding region operatively linked to *trp* transcription control sequences and to a fusion sequence encoding a poly-histidine segment comprising 6 histidines is produced in the following manner. Nucleic acid molecule nCtCal$_{1212}$ spanning the flea calreticulin gene from the putative start through stop codons is produced by PCR amplification of the TA Vector® clone containing nCtCal$_{1218}$ (produced as described in Example 5) using the following primers: Cal-S, a flea calreticulin sense primer having SEQ ID NO:21, namely 5' GAGCTCTCGAGAATAAATATGAAAGCAATTTTG 3' ; and Cal-A, a flea calreticulin anti-sense primer having

SEQ ID NO:22, namely 5' GGACCTCGAGAATCACAGTTCATCGTGCTCAGC 3'. The PCR product is digested with Xho1 restriction endonuclease, gel purified and subcloned into expression vector $TrpT^2ori$/T7-RSET-B (produced as described in PCT Publication No. WO 95/24198, published September 14, 1995, by Tripp et al.), that has been cleaved with Xho1. The resulting recombinant molecule, denoted pTrpHis-nCtCal$_{1212}$, is transformed into *E. coli* to form recombinant cell *E. coli*:pTrpHis-nCtCal$_{1212}$. Such a recombinant cell, when cultured under effective conditions such as those described in Example 4, leads to the production of a fusion protein denoted herein as PHIS-PCtCal$_{403}$.

SEQUENCE LISTING

The following Sequence Listing is submitted pursuant to 37 CFR §1.821. A copy in computer readable form is also submitted herewith.

Applicants assert pursuant to 37 CFR §1.821(f) that the content of the paper and computer readable copies of SEQ ID NO:1 through SEQ ID NO:22 submitted herewith are the same.

(1) GENERAL INFORMATION:

(i) APPLICANT: Stiegler, Gary L.
Rushlow, Keith E.

(ii) TITLE OF INVENTION: HEMATOPHAGOUS INSECT CALRETICULIN NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 22

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Sheridan Ross & McIntosh
(B) STREET: 1700 Lincoln Street, Suite 3500
(C) CITY: Denver
(D) STATE: Colorado
(E) COUNTRY: U.S.A.
(F) ZIP: 80203

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Connell, Gary J.
(B) REGISTRATION NUMBER: 32,020
(C) REFERENCE/DOCKET NUMBER: 2618-17-C1PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (303) 863-9700
(B) TELEFAX: (303) 863-0223

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 257 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..255

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
AAA CCA GAA GAC TGG GAC AAC CGT GCA ACT ATT CCT GAC CCT AAT GAC      48
Lys Pro Glu Asp Trp Asp Asn Arg Ala Thr Ile Pro Asp Pro Asn Asp
 1               5                  10                  15

ACT AAA CCG GAA GAT TGG GAC AAG CCA GAA CAC ATT CCT GAT CCT GAT      96
Thr Lys Pro Glu Asp Trp Asp Lys Pro Glu His Ile Pro Asp Pro Asp
            20                  25                  30

GCT ACC AAA CCT GAT GAT TGG GAT GAA GAG ATG GAT GGT GAA TGG GAA     144
Ala Thr Lys Pro Asp Asp Trp Asp Glu Glu Met Asp Gly Glu Trp Glu
        35                  40                  45

CCT GCT ATG ATT GAC AAC CCT GAA TAT AAG GGA GAA TGG GCA CCA AAA     192
Pro Ala Met Ile Asp Asn Pro Glu Tyr Lys Gly Glu Trp Ala Pro Lys
    50                  55                  60

CAG ATT GAC AAT CCT GAC TAC AAA GGT GTA TGG GTT CAC CCT GAG ATA     240
Gln Ile Asp Asn Pro Asp Tyr Lys Gly Val Trp Val His Pro Glu Ile
65                  70                  75                  80

GAT AAT CCT GAG TAT AC                                              257
Asp Asn Pro Glu Tyr
                85
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Lys Pro Glu Asp Trp Asp Asn Arg Ala Thr Ile Pro Asp Pro Asn Asp
1 5 10 15

Thr Lys Pro Glu Asp Trp Asp Lys Pro Glu His Ile Pro Asp Pro Asp
20 25 30

Ala Thr Lys Pro Asp Asp Trp Asp Glu Glu Met Asp Gly Glu Trp Glu
35 40 45

Pro Ala Met Ile Asp Asn Pro Glu Tyr Lys Gly Glu Trp Ala Pro Lys
50 55 60

Gln Ile Asp Asn Pro Asp Tyr Lys Gly Val Trp Val His Pro Glu Ile
65 70 75 80

Asp Asn Pro Glu Tyr
85

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..23
(D) OTHER INFORMATION: /label= PRIMER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AARCCNGARG AYTGGGAYAA RCC 23

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..23
(D) OTHER INFORMATION: /label= PRIMER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTRTAYTCNG GRTTRTCDAT YTC 23

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..20
(D) OTHER INFORMATION: /label= PRIMER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAYCCNGARG GNGARTGGAA 20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /label= PRIMER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAAACAGCT ATGACCATG 19

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..25
(D) OTHER INFORMATION: /label= PRIMER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGAACCAGAC ACCTTTGTAG TCAGG 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= PRIMER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTAAAACGAC GGCCAGT 17

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..23
(D) OTHER INFORMATION: /label= PRIMER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGAAGATTGG GACAAGCCAG AAC 23

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..24
(D) OTHER INFORMATION: /label= PRIMER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTGGGACAAG CCAGAACACA TTCC 24

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1589 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 151..1360

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGCACGAGCG ACGACGAATG CATAAGCCGC ATTTGTTATT GAGAAATATA TGCTAAAATA 60

TATTGTCTCA GAATTATTAT AATGTGCTGA GTTTGCATTA ATATTGAAAC GTTTTATTCA 120

TTCTGAGGCA TCTATATAAA ATAAATAAAT ATG AAA GCA ATT TTG ATA ACA TTG 174
Met Lys Ala Ile Leu Ile Thr Leu
1 5

ATA GTC GCC GCG GCT GTG TAT TCC GTA AGG CCT GAG GTT TTC CTG GAA 222
Ile Val Ala Ala Ala Val Tyr Ser Val Arg Pro Glu Val Phe Leu Glu
10 15 20

GAA AAC TTC GTA GAC GAT ACG TGG ACA AAT ACA TGG GTT TAT AGT GAA 270
Glu Asn Phe Val Asp Asp Thr Trp Thr Asn Thr Trp Val Tyr Ser Glu
25 30 35 40

CAC CCT GGC AAA GAA TTC GGC AAA TTC GTG CAC ACT GCC GGA AAG TTC 318
His Pro Gly Lys Glu Phe Gly Lys Phe Val His Thr Ala Gly Lys Phe
45 50 55

TAT AAC GAT GCC GAA GCA GAC AAA GGT TTG CAA ACA GGT CAA GAT GCT 366
Tyr Asn Asp Ala Glu Ala Asp Lys Gly Leu Gln Thr Gly Gln Asp Ala
60 65 70

AGG TTC TAC GCT CTA TCT CAT AAG TTC AAA CCT TTC TCA AAT AAA GAC 414
Arg Phe Tyr Ala Leu Ser His Lys Phe Lys Pro Phe Ser Asn Lys Asp
75 80 85

```
AAG ACA TTA GTT GTA CAA TTT TCC GTT AAA CAT GAA CAA AAC ATT GAC      462
Lys Thr Leu Val Val Gln Phe Ser Val Lys His Glu Gln Asn Ile Asp
 90                  95                 100

TGT GGA GGT GGT TAC TTG AAG GGT TTC GAA TTC AGT GTG AAT CAA AAG      510
Cys Gly Gly Gly Tyr Leu Lys Gly Phe Glu Phe Ser Val Asn Gln Lys
105                 110                 115                 120

GAC ATG CAT GGG GAA AGT CCC TAT GAA ATT ATG TTT GGT CCT GAC ATT      558
Asp Met His Gly Glu Ser Pro Tyr Glu Ile Met Phe Gly Pro Asp Ile
                125                 130                 135

TGT GAC CCA GGA ACT AAG AAG GTT CAC GTA ATC TTC AGC TAC AAG GGT      606
Cys Asp Pro Gly Thr Lys Lys Val His Val Ile Phe Ser Tyr Lys Gly
            140                 145                 150

AAA AAT GTT TTG ATC AAT AAG GAT ATC CGC TGC AAA GAT GAT GTC TAT      654
Lys Asn Val Leu Ile Asn Lys Asp Ile Arg Cys Lys Asp Asp Val Tyr
        155                 160                 165

ACT CAT GTA TAC ACT TTG GTT GTT AAG CCC GAT AAT ACC TAT GAG GTG      702
Thr His Val Tyr Thr Leu Val Val Lys Pro Asp Asn Thr Tyr Glu Val
    170                 175                 180

TTG ATT GAT AAT GAG AAG GTT GAA AGT GGT AAC TTG GAA GAT GAC TGG      750
Leu Ile Asp Asn Glu Lys Val Glu Ser Gly Asn Leu Glu Asp Asp Trp
185                 190                 195                 200

GAA TTC CTA GCC CCC AAG AAA ATC AAG GAT CCA GAA GCT AAA AAA CCA      798
Glu Phe Leu Ala Pro Lys Lys Ile Lys Asp Pro Glu Ala Lys Lys Pro
                205                 210                 215

GCA GAT TGG GAT GAG CGT GCA ACT ATT CCT GAC CCT AAT GAC ACC AAA      846
Ala Asp Trp Asp Glu Arg Ala Thr Ile Pro Asp Pro Asn Asp Thr Lys
            220                 225                 230

CCT GAA GAT TGG GAC AAG CCA GAA CAC ATT CCT GAT CCT GAT GCT ACC      894
Pro Glu Asp Trp Asp Lys Pro Glu His Ile Pro Asp Pro Asp Ala Thr
        235                 240                 245

AAA CCT GAT GAC TGG GAT GAA GAG ATG GAT GGT GAA TGG GAA CCT GCT      942
Lys Pro Asp Asp Trp Asp Glu Glu Met Asp Gly Glu Trp Glu Pro Ala
    250                 255                 260

ATG ATT GAC AAC CCT GAA TAT AAG GGA GAA TGG GCA CCA AAA CAG ATT      990
Met Ile Asp Asn Pro Glu Tyr Lys Gly Glu Trp Ala Pro Lys Gln Ile
265                 270                 275                 280

GAC AAT CCT GAC TAC AAA GGT GTC TGG GTT CAC CCT GAA ATT GAT AAT     1038
Asp Asn Pro Asp Tyr Lys Gly Val Trp Val His Pro Glu Ile Asp Asn
                285                 290                 295

CCA GAA TAT GTT CCT GAT ACT CAA CTT TAC AAA CGT GAT GAG ATT TGT     1086
Pro Glu Tyr Val Pro Asp Thr Gln Leu Tyr Lys Arg Asp Glu Ile Cys
            300                 305                 310

GCC ATT GGT TTA GAT TTA TGG CAA GTA AAG GCT GGA ACA ATA TTC GAC     1134
Ala Ile Gly Leu Asp Leu Trp Gln Val Lys Ala Gly Thr Ile Phe Asp
        315                 320                 325

AAT ATT TTA ATC ACA GAT GAT GTT GAT TAT GCA AAG AAA ATA GCA GAA     1182
Asn Ile Leu Ile Thr Asp Asp Val Asp Tyr Ala Lys Lys Ile Ala Glu
    330                 335                 340

GGT GTT AAA TCT ACC CAG GAA GGA GAA AAG AAA ATG AAA GAT GCT CAA     1230
Gly Val Lys Ser Thr Gln Glu Gly Glu Lys Lys Met Lys Asp Ala Gln
345                 350                 355                 360
```

```
GAT GAA GAA GAA AGG AAA GCC AGG GAA GCC GAA ACA AAA GAA GAA AAT    1278
Asp Glu Glu Glu Arg Lys Ala Arg Glu Ala Glu Thr Lys Glu Glu Asn
                365                 370                 375

GAC ACA GAT GCT GAT GAA GAC TTA GAT GAT AAT GCC GAA ACA CCA GAA    1326
Asp Thr Asp Ala Asp Glu Asp Leu Asp Asp Asn Ala Glu Thr Pro Glu
            380                 385                 390

GAA GAC ACA CTC GAT GCT GAG CAC GAT GAA CTG T GATTTTTAAG           1370
Glu Asp Thr Leu Asp Ala Glu His Asp Glu Leu
        395                 400

TGCTACTCAC CATAAACTTT TCACATTGGC TTAATTTATT TCCGTTAAAT CATCCAACAT  1430

CTATACATTA ATTATTACCT TGTAGAAAAT TGTGTTTGTG AAAAATTTGT CTCCGTTTAC  1490

TTGAAACAAT GAAGTGCATG CCAATTGTGT AATAATCGAC TGTGCCCAAA ATAAATTATT  1550

TAATTCTTGT TCAATAAGAT TTTGTTATAC GTAAGTTTT                         1589
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 403 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Lys Ala Ile Leu Ile Thr Leu Ile Val Ala Ala Ala Val Tyr Ser
  1               5                  10                  15

Val Arg Pro Glu Val Phe Leu Glu Glu Asn Phe Val Asp Asp Thr Trp
            20                  25                  30

Thr Asn Thr Trp Val Tyr Ser Glu His Pro Gly Lys Glu Phe Gly Lys
        35                  40                  45

Phe Val His Thr Ala Gly Lys Phe Tyr Asn Asp Ala Glu Ala Asp Lys
    50                  55                  60

Gly Leu Gln Thr Gly Gln Asp Ala Arg Phe Tyr Ala Leu Ser His Lys
65                  70                  75                  80

Phe Lys Pro Phe Ser Asn Lys Asp Lys Thr Leu Val Val Gln Phe Ser
                85                  90                  95

Val Lys His Glu Gln Asn Ile Asp Cys Gly Gly Gly Tyr Leu Lys Gly
            100                 105                 110

Phe Glu Phe Ser Val Asn Gln Lys Asp Met His Gly Glu Ser Pro Tyr
        115                 120                 125

Glu Ile Met Phe Gly Pro Asp Ile Cys Asp Pro Gly Thr Lys Lys Val
    130                 135                 140

His Val Ile Phe Ser Tyr Lys Gly Lys Asn Val Leu Ile Asn Lys Asp
145                 150                 155                 160

Ile Arg Cys Lys Asp Asp Val Tyr Thr His Val Tyr Thr Leu Val Val
                165                 170                 175
```

Lys Pro Asp Asn Thr Tyr Glu Val Leu Ile Asp Asn Glu Lys Val Glu
180 185 190

Ser Gly Asn Leu Glu Asp Asp Trp Glu Phe Leu Ala Pro Lys Lys Ile
195 200 205

Lys Asp Pro Glu Ala Lys Lys Pro Ala Asp Trp Asp Glu Arg Ala Thr
210 215 220

Ile Pro Asp Pro Asn Asp Thr Lys Pro Glu Asp Trp Asp Lys Pro Glu
225 230 235 240

His Ile Pro Asp Pro Asp Ala Thr Lys Pro Asp Asp Trp Asp Glu Glu
245 250 255

Met Asp Gly Glu Trp Glu Pro Ala Met Ile Asp Asn Pro Glu Tyr Lys
260 265 270

Gly Glu Trp Ala Pro Lys Gln Ile Asp Asn Pro Asp Tyr Lys Gly Val
275 280 285

Trp Val His Pro Glu Ile Asp Asn Pro Glu Tyr Val Pro Asp Thr Gln
290 295 300

Leu Tyr Lys Arg Asp Glu Ile Cys Ala Ile Gly Leu Asp Leu Trp Gln
305 310 315 320

Val Lys Ala Gly Thr Ile Phe Asp Asn Ile Leu Ile Thr Asp Asp Val
325 330 335

Asp Tyr Ala Lys Lys Ile Ala Glu Gly Val Lys Ser Thr Gln Glu Gly
340 345 350

Glu Lys Lys Met Lys Asp Ala Gln Asp Glu Glu Glu Arg Lys Ala Arg
355 360 365

Glu Ala Glu Thr Lys Glu Glu Asn Asp Thr Asp Ala Asp Glu Asp Leu
370 375 380

Asp Asp Asn Ala Glu Thr Pro Glu Glu Asp Thr Leu Asp Ala Glu His
385 390 395 400

Asp Glu Leu

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1589 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAAACTTACG TATAACAAAA TCTTATTGAA CAAGAATTAA ATAATTTATT TTGGGCACAG 60
TCGATTATTA CACAATTGGC ATGCACTTCA TTGTTTCAAG TAAACGGAGA CAAATTTTTC 120
ACAAACACAA TTTTCTACAA GGTAATAATT AATGTATAGA TGTTGGATGA TTTAACGGAA 180
ATAAATTAAG CCAATGTGAA AAGTTTATGG TGAGTAGCAC TTAAAAATCA CAGTTCATCG 240
TGCTCAGCAT CGAGTGTGTC TTCTTCTGGT GTTTCGGCAT TATCATCTAA GTCTTCATCA 300
GCATCTGTGT CATTTTCTTC TTTTGTTTCG GCTTCCCTGG CTTTCCTTTC TTCTTCATCT 360
TGAGCATCTT TCATTTTCTT TTCTCCTTCC TGGGTAGATT TAACACCTTC TGCTATTTTC 420
TTTGCATAAT CAACATCATC TGTGATTAAA ATATTGTCGA ATATTGTTCC AGCCTTTACT 480
TGCCATAAAT CTAAACCAAT GGCACAAATC TCATCACGTT TGTAAAGTTG AGTATCAGGA 540
ACATATTCTG GATTATCAAT TTCAGGGTGA ACCCAGACAC CTTTGTAGTC AGGATTGTCA 600
ATCTGTTTTG GTGCCCATTC TCCCTTATAT TCAGGGTTGT CAATCATAGC AGGTTCCCAT 660
TCACCATCCA TCTCTTCATC CCAGTCATCA GGTTTGGTAG CATCAGGATC AGGAATGTGT 720
TCTGGCTTGT CCCAATCTTC AGGTTTGGTG TCATTAGGGT CAGGAATAGT TGCACGCTCA 780
TCCCAATCTG CTGGTTTTTT AGCTTCTGGA TCCTTGATTT TCTTGGGGGC TAGGAATTCC 840
CAGTCATCTT CCAAGTTACC ACTTTCAACC TTCTCATTAT CAATCAACAC CTCATAGGTA 900
TTATCGGGCT TAACAACCAA AGTGTATACA TGAGTATAGA CATCATCTTT GCAGCGGATA 960
TCCTTATTGA TCAAAACATT TTTACCCTTG TAGCTGAAGA TTACGTGAAC CTTCTTAGTT 1020
CCTGGGTCAC AAATGTCAGG ACCAAACATA ATTTCATAGG GACTTTCCCC ATGCATGTCC 1080
TTTTGATTCA CACTGAATTC GAAACCCTTC AAGTAACCAC CTCCACAGTC AATGTTTTGT 1140
TCATGTTTAA CGGAAAATTG TACAACTAAT GTCTTGTCTT TATTTGAGAA AGGTTTGAAC 1200
TTATGAGATA GAGCGTAGAA CCTAGCATCT TGACCTGTTT GCAAACCTTT GTCTGCTTCG 1260
GCATCGTTAT AGAACTTTCC GGCAGTGTGC ACGAATTTGC CGAATTCTTT GCCAGGGTGT 1320
TCACTATAAA CCCATGTATT TGTCCACGTA TCGTCTACGA AGTTTTCTTC CAGGAAAACC 1380
TCAGGCCTTA CGGAATACAC AGCCGCGGCG ACTATCAATG TTATCAAAAT TGCTTTCATA 1440
TTTATTTATT TTATATAGAT GCCTCAGAAT GAATAAAACG TTTCAATATT AATGCAAACT 1500
CAGCACATTA TAATAATTCT GAGACAATAT ATTTTAGCAT ATATTTCTCA ATAACAAATG 1560
CGGCTTATGC ATTCGTCGTC GCTCGTGCC 1589

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1209 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGAAAGCAA TTTTGATAAC ATTGATAGTC GCCGCGGCTG TGTATTCCGT AAGGCCTGAG 60

GTTTTCCTGG AAGAAAACTT CGTAGACGAT ACGTGGACAA ATACATGGGT TTATAGTGAA 120

CACCCTGGCA AAGAATTCGG CAAATTCGTG CACACTGCCG GAAAGTTCTA TAACGATGCC 180

GAAGCAGACA AAGGTTTGCA AACAGGTCAA GATGCTAGGT TCTACGCTCT ATCTCATAAG 240

TTCAAACCTT TCTCAAATAA AGACAAGACA TTAGTTGTAC AATTTTCCGT TAAACATGAA 300

CAAAACATTG ACTGTGGAGG TGGTTACTTG AAGGGTTTCG AATTCAGTGT GAATCAAAAG 360

GACATGCATG GGGAAAGTCC CTATGAAATT ATGTTTGGTC CTGACATTTG TGACCCAGGA 420

ACTAAGAAGG TTCACGTAAT CTTCAGCTAC AAGGGTAAAA ATGTTTTGAT CAATAAGGAT 480

ATCCGCTGCA AAGATGATGT CTATACTCAT GTATACACTT TGGTTGTTAA GCCCGATAAT 540

ACCTATGAGG TGTTGATTGA TAATGAGAAG GTTGAAAGTG GTAACTTGGA AGATGACTGG 600

GAATTCCTAG CCCCCAAGAA AATCAAGGAT CCAGAAGCTA AAAAACCAGC AGATTGGGAT 660

GAGCGTGCAA CTATTCCTGA CCCTAATGAC ACCAAACCTG AAGATTGGGA CAAGCCAGAA 720

CACATTCCTG ATCCTGATGC TACCAAACCT GATGACTGGG ATGAAGAGAT GGATGGTGAA 780

TGGGAACCTG CTATGATTGA CAACCCTGAA TATAAGGGAG AATGGGCACC AAAACAGATT 840

GACAATCCTG ACTACAAAGG TGTCTGGGTT CACCCTGAAA TTGATAATCC AGAATATGTT 900

CCTGATACTC AACTTTACAA ACGTGATGAG ATTTGTGCCA TTGGTTTAGA TTTATGGCAA 960

GTAAAGGCTG GAACAATATT CGACAATATT TTAATCACAG ATGATGTTGA TTATGCAAAG 1020

AAAATAGCAG AAGGTGTTAA ATCTACCCAG GAAGGAGAAA AGAAAATGAA AGATGCTCAA 1080

GATGAAGAAG AAAGGAAAGC CAGGGAAGCC GAAACAAAAG AAGAAAATGA CACAGATGCT 1140

GATGAAGACT TAGATGATAA TGCCGAAACA CCAGAAGAAG ACACACTCGA TGCTGAGCAC 1200

GATGAACTG 1209

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1209 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGTTCATCG TGCTCAGCAT CGAGTGTGTC TTCTTCTGGT GTTTCGGCAT TATCATCTAA 60

GTCTTCATCA GCATCTGTGT CATTTTCTTC TTTTGTTTCG GCTTCCCTGG CTTTCCTTTC 120

TTCTTCATCT TGAGCATCTT TCATTTTCTT TTCTCCTTCC TGGGTAGATT TAACACCTTC 180

TGCTATTTTC TTTGCATAAT CAACATCATC TGTGATTAAA ATATTGTCGA ATATTGTTCC 240

AGCCTTTACT TGCCATAAAT CTAAACCAAT GGCACAAATC TCATCACGTT TGTAAAGTTG 300

AGTATCAGGA ACATATTCTG GATTATCAAT TTCAGGGTGA ACCCAGACAC CTTTGTAGTC 360

AGGATTGTCA ATCTGTTTTG GTGCCCATTC TCCCTTATAT TCAGGGTTGT CAATCATAGC 420

AGGTTCCCAT TCACCATCCA TCTCTTCATC CCAGTCATCA GGTTTGGTAG CATCAGGATC 480

AGGAATGTGT TCTGGCTTGT CCCAATCTTC AGGTTTGGTG TCATTAGGGT CAGGAATAGT 540

TGCACGCTCA TCCCAATCTG CTGGTTTTTT AGCTTCTGGA TCCTTGATTT TCTTGGGGGC 600

TAGGAATTCC CAGTCATCTT CCAAGTTACC ACTTTCAACC TTCTCATTAT CAATCAACAC 660

CTCATAGGTA TTATCGGGCT TAACAACCAA AGTGTATACA TGAGTATAGA CATCATCTTT 720

GCAGCGGATA TCCTTATTGA TCAAAACATT TTTACCCTTG TAGCTGAAGA TTACGTGAAC 780

CTTCTTAGTT CCTGGGTCAC AAATGTCAGG ACCAAACATA ATTTCATAGG GACTTTCCCC 840

ATGCATGTCC TTTTGATTCA CACTGAATTC GAAACCCTTC AAGTAACCAC CTCCACAGTC 900

AATGTTTTGT TCATGTTTAA CGGAAAATTG TACAACTAAT GTCTTGTCTT TATTTGAGAA 960

AGGTTTGAAC TTATGAGATA GAGCGTAGAA CCTAGCATCT TGACCTGTTT GCAAACCTTT 1020

GTCTGCTTCG GCATCGTTAT AGAACTTTCC GGCAGTGTGC ACGAATTTGC CGAATTCTTT 1080

GCCAGGGTGT TCACTATAAA CCCATGTATT TGTCCACGTA TCGTCTACGA AGTTTTCTTC 1140

CAGGAAAACC TCAGGCCTTA CGGAATACAC AGCCGCGGCG ACTATCAATG TTATCAAAAT 1200

TGCTTTCAT 1209

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 257 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTATACTCTG GATTATCTAT TTCAGGGTGA ACCCAGATAC CTTTGTAGTC AGGATTGTCA 60

ATCTGTTTTG GTGCCCATTC TCCCTTATAT TCAGGGTTGT CAATCATAGC AGGTTCCCAT 120

TCACCATCCA TCTCTTCATC CCAATCATCA GGTTTGGTAG CATCAGGATC AGGAATGTGT 180

TCTGGCTTGT CCCAATCTTC CGGTTTAGTG TCATTAGGGT CAGGAATAGT TGCACGGTTG 240

TCCCAGTCTT CTGGTTT 257

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATTAGGGTCA GGAATAGTTG CACGCTC 27

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..25
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CATGTATACA CTTTGGTTGT TAAGC 25

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATAAATATGA AAGCAATTTT GATAACA 27

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..30
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TCACAGTTCA TCGTGCTCAG CATCGAGTGT 30

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..33
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GAGCTCTCGA GAATAAATAT GAAAGCAATT TTG 33

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..33
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGACCTCGAG AATCACAGTT CATCGTGCTC AGC 33

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

What is claimed is:

1. An isolated hematophagous insect calreticulin protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a flea calreticulin gene.

2. The protein of Claim 1, wherein said flea calreticulin gene comprises nucleic acid sequence SEQ ID NO:1.

3. The protein of Claim 1, wherein said flea calreticulin gene encodes a protein comprising amino acid sequence SEQ ID NO:2.

4. The protein of Claim 1, wherein said protein can be isolated from insect saliva.

5. The protein of Claim 1, wherein said protein comprises an amino acid sequence that is at least about 85% identical to an amino acid sequence represented by an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:12.

6. The protein of Claim 1, wherein said protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:12.

7. The protein of Claim 1, wherein said protein is encoded by a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11 and SEQ ID NO:14.

8. The protein of Claim 1, wherein said protein, when administered to an animal, is capable of reducing insect infestation by a method comprising altering the

blood feeding behavior of insects that feed from said treated animal.

9. The protein of Claim 1, wherein said protein, when administered to an animal, reduces calreticulin activity in insects feeding from said animal, thereby reducing insect burden on said animal and in the environment of said animal.

10. The protein of Claim 1, wherein said protein, when administered to an animal, is capable of eliciting an immune response against an insect calreticulin.

11. The protein of Claim 1, wherein said protein, when administered to an animal, is capable of substantially desensitizing said animal to allergic dermatitis.

12. The protein of Claim 11, wherein said allergic dermatitis is selected from the group consisting of flea allergic dermatitis, mosquito allergic dermatitis and *Culicoides* allergic dermatitis.

13. The protein of Claim 1, wherein said protein is used to identify animals susceptible to or having allergic dermatitis.

14. An isolated antibody capable of selectively binding to an isolated protein as set forth in Claim 1.

15. An isolated hematophagous insect calreticulin nucleic acid molecule that hybridizes under stringent hybridization conditions to a flea calreticulin gene.

16. The nucleic acid molecule of Claim 15, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions to a nucleic acid sequence

selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16.

17. The nucleic acid molecule of Claim 15, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions to a nucleic acid sequence encoding a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:12.

18. The nucleic acid molecule of Claim 15, wherein said nucleic acid molecule comprises a nucleic acid sequence having at least about 80% identity with nucleic acid sequence SEQ ID NO:1.

19. The nucleic acid molecule of Claim 15, wherein said nucleic acid molecule comprises an oligonucleotide.

20. The nucleic acid molecule of Claim 15, wherein said nucleic acid sequence encodes a hematophagous insect calreticulin protein having calcium binding activity.

21. The nucleic acid molecule of Claim 15 or the protein of Claim 1, wherein said insect is selected from the group consisting of fleas, midges, mosquitos, sand flies, black flies, horse flies, horn flies, deer flies, tsetse flies, buffalo flies, blow flies, stable flies, myiasis-causing flies, biting gnats, lice and true bugs.

22. The nucleic acid molecule of Claim 15 or the protein of Claim 1, wherein said insect is a flea of a genus selected from the group consisting of *Ctenocephalides*, *Cyopsyllus*, *Diamanus*, *Echidnophaga*,

Nosopsyllus, *Pulex*, *Tunga*, *Oropsylla*, *Orchopeus* and *Xenopsylla*.

23. The nucleic acid molecule of Claim 15 or the protein of Claim 1,, wherein said insect is a flea of a species selected from the group consisting of *Ctenocephalides felis*, *Ctenocephalides canis*, *Pulex irritans*, *Oropsylla (Thrassis) bacchi*, *Oropsylla (Diamanus) montana*, *Orchopeus howardi*, *Xenopsylla cheopis* and *Pulex simulans*.

24. The nucleic acid molecule of Claim 15, wherein said nucleic acid molecule encodes a protein capable of eliciting an immune response against a hematophagous insect calreticulin.

25. The nucleic acid molecule of Claim 15, wherein said nucleic acid molecule, when administered to an animal, reduces calreticulin activity in insects feeding from said animal, thereby reducing insect burden on said animal and in the environment of said animal.

26. The nucleic acid molecule of Claim 15, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16.

27. The nucleic acid molecule of Claim 15, wherein said nucleic acid molecule encodes a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:12.

28. A recombinant molecule comprising an isolated

nucleic acid molecule as set forth in Claim 15 operatively linked to a transcription control sequence.

29. A recombinant cell comprising a cell having an isolated nucleic acid molecule as set forth in Claim 15, said cell being capable of expressing said nucleic acid molecule.

30. The nucleic acid molecule of Claim 15, wherein said nucleic acid molecule comprises a nucleic acid molecule selected from the group consisting of $nCtCal_{257}$, $nCtCal_{850}$, $nCtCal_{550}$, $nCtCal_{556}$, $nCtCal_{1589}$, $nCtCal_{1209}$, $nCtCal_{1212}$, $nCtCal_{665}$, $nCtCal_{750}$, and $nCtCal_{1218}$.

31. A therapeutic composition for protecting an animal from hematophagous insect infestation, said composition comprising a compound selected from the group consisting of an isolated hematophagous insect calreticulin protein or a mimetope thereof, an isolated nucleic acid molecule capable of hybridizing under stringent hybridization conditions with a flea calreticulin gene, an isolated anti-hematophagous insect calreticulin antibody or a mimetope thereof, a calreticulin inhibitory compound and a mixture thereof, said composition, when administered to an animal, being able to reduce hematophagous insect burden on said animal and in the environment of said animal.

32. The composition of Claim 31, wherein said animal is selected from the group consisting of mammals and birds.

33. The composition of Claim 31, wherein said animal is selected from the group consisting of cats, dogs, sheep, cows, pigs, horses and goats.

34. A method to protect an animal from hematophagous insect infestation, comprising treating an animal with a therapeutic composition that includes a compound selected from the group consisting of an isolated hematophagous insect calreticulin protein or a mimetope thereof, an isolated nucleic acid molecule capable of hybridizing under stringent hybridization conditions with a flea calreticulin gene, an isolated anti-hematophagous insect calreticulin antibody or a mimetope thereof, a calreticulin inhibitory compound, and a mixture thereof, said composition, when administered to an animal, being able to reduce hematophagous insect burden on said animal and in the environment of said animal.

35. The method of Claim 34 or the therapeutic composition of Claim 31, wherein said composition further comprises a component selected from the group consisting of a pharmaceutically acceptable excipient, an adjuvant, a carrier, and a mixture thereof.

36. A method to produce a hematophagous insect calreticulin protein comprising culturing in an effective medium a recombinant cell transformed with a nucleic acid molecule encoding said protein to produce said protein.

37. The method of Claim 36, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11 and SEQ ID NO:14.

38. The method of Claim 36, wherein said nucleic acid molecule encodes a protein comprising an amino acid

sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:12.

39. A method to identify a compound capable of inhibiting hematophagous insect calreticulin activity, said method comprising:

(a) contacting an isolated hematophagous insect calreticulin protein with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has calreticulin activity; and

(b) determining if said putative inhibitory compound inhibits said calreticulin activity.

40. A test kit to identify a compound capable of inhibiting hematophagous insect calreticulin activity, said test kit comprising an isolated hematophagous insect calreticulin protein having calreticulin activity and a means for determining the extent of inhibition of said activity in the presence of a putative inhibitory compound.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03133

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/185.1, 275.1; 435/69.1, 252.3, 240.1, 320.1; 514/2, 12; 530/300, 324, 858; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, MEDLINE, EMBASE
search terms: flea, calreticulin, antigen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	K.K. Murthy et al. Structural homology between the rat calreticulin gene produce and the Onchocerca volvulus antigen Ral-1. Nucleic Acids Res. 1990, Vol. 18, No. 16, page 4933, see entire document.	1, 14-17, 19, 20, 24, 28, 29, 46
Y	M.J. Smith et al. Multiple zones in the sequence of calreticulin (CRP55, calregulin, HACBP), a major calcium binding ER/SR protein. EMBO Journal. 1989, Vol. 8, Nol. 12, pages 3581-3586, especially abstract, figure 2.	1, 14-17, 19, 20, 24, 28, 29, 36
A	Journal of Cellular Biochemistry Supp., Vol. 0, No. 21A, issued 1995, abstract no. C3-213, Jaworski, D. C. "Characterization of calreticulin in cat fleas".	1-40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 JUNE 1996	Date of mailing of the international search report 13 JUN 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DIAN C. JACOBSON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03133

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07K 14/09, 14/435, 16/18; C12N 1/21, 15/09, 15/12, 15/63; A61K 35/64, 38/16, 39/00, 39/35

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/185.1, 275.1; 435/69.1, 252.3, 240.1, 320.1; 514/2, 12; 530/300, 324, 858; 536/23.5